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Nitric oxide mediates Cd-dependent induction of signaling–associated genes

Jagna Chmielowska-Bąk and Joanna Deckert*

Department of Plant Ecophysiology; Institute of Experimental Biology, Faculty of Biology; Adam Mickiewicz University; Poznań, Poland

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The first reaction of plants to stress factors, including cadmium, is activation of signal transduction pathways leading to the mobilization of defense mechanisms. In a recent study we have demonstrated that cadmium causes a rapid induction of several genes associated with cellular signaling in soybean seedlings. Here we show that nitric oxide can be an important signaling molecule mediating observed increase in genes expression under short-term cadmium stress.

Cadmium is a heavy metal that exhibits deleterious effect in all organisms. The earliest response to this stress factor is activation of signal transduction pathways leading to the changes in genes expression crucial for acquisition of tolerance. In a recent study[1] we have shown that cadmium causes increase in expression of several signaling-associated genes in the roots of soybean seedlings. A Cd-dependent induction of genes encoding 1-aminocyclopropane-1-carboxylic acid synthase (ACS), mitogen-activated protein kinase2 (MAPKK2), and DOF1 and MYBZ2 transcription factors was noted after 3 h, while 6 h-long treatment led to the increase in the levels of mRNA encoding nitrate reductase (NR), ACS, MYBZ2, and bZIP62 transcription factors.[1] The obtained results raise the question about signaling molecules mediating the rapid Cd-dependent induction of analyzed genes.

One of the potential candidates is nitric oxide, an important signaling molecule involved in the regulation of the expression of numerous genes.[2-4] Increase in NO levels in response to short-term cadmium stress has been observed in various plant species.[4-8] Moreover, it has been shown that nitric oxide is engaged in stimulation of cadmium uptake,[4-6] mediation of Cd-dependent programmed cell death,[5,6] and regulation of antioxidant enzymes activity,[9,10] reactive oxygen species levels,[9] and phytochelatin synthesis[10] in plants exposed to cadmium.

The results of the present study show that treatment with nitric oxide scavenger, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (PTIO), reversed the observed increase in expression of all genes induced by cadmium stress in the roots of soybean seedlings after 3 h of treatment (**Fig. 1A-D**). Interestingly, in the case of genes induced after 6 h of cadmium application, this effect was not observed (**Fig. 1E-H**). In order to examine if the impact of PTIO on genes expression depended on changes in Cd uptake, Cd accumulation has been evaluated in the roots of soybean seedlings. Histochemical staining with Dithizone reagent showed that after 3 h of treatment PTIO slightly increased the amount of accumulated metal (**Figs. 2A** and **3A**), while after longer treatment periods (6 h) it strongly attenuated Cd uptake (**Figs. 2B** and **3B**). The obtained results suggest that, after short treatment periods (3 h), NO mediates Cd-dependent induction of several genes associated with signaling pathways.

On the basis of presented research, earlier studies performed in our laboratory, and literature data, it can be concluded that early signaling events triggered by cadmium stress involve accumulation of NO[11] mediating induction of *ACS*, *MAPKK2*, *DOF1*, and *MYBZ2* genes. Exposure to Cd also causes rapid release of Ca^{2+} to cytoplasm[12,13] leading to the activation of NADPH oxidase and generation of reactive oxygen species (ROS).[12] All mentioned signaling molecules, NO, Ca^{2+}, and ROS, are engaged in the stimulation of MAPK cascades observed in response to short-term cadmium stress on both transcription and post-transcription levels.[1,14-17] Increase in *ACS* expression in turn leads to the induction of ethylene biosynthesis.[1] The rapid response to Cd stress also includes stimulation of several transcription factors[18] among others DOF1, MYBZ2, and bZIP62.[1]

Disclosure of Potential Conflicts of Interests

No potential conflicts of interests were disclosed.

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*Correspondence to: Joanna Deckert; Email: joanna.deckert@amu.edu.pl

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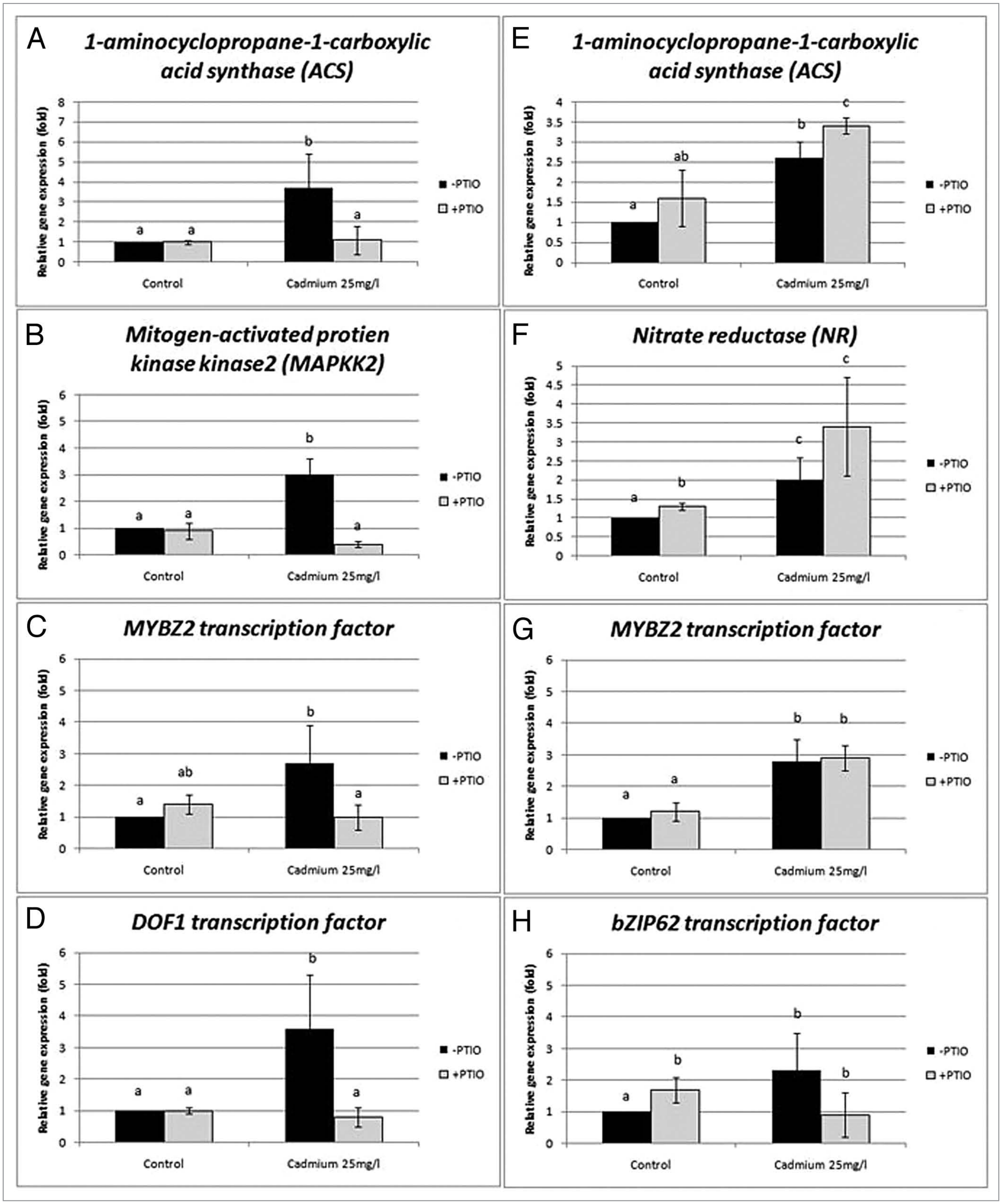

Figure 1. Relative expression of Cd-inducible genes after 3 (**A**-**D**) and 6 (**E**-**H**) hours of treatment with distilled water (control), 50μM PTIO, $CdCl_2$ with cadmium in the concentration of 25 mg L-1 (corresponding to 223μM), or $CdCl_2$ with cadmium in the concentration of 25 mg L-1 and 50μM PTIO. Genes expression has been measured with the use of real-time PCR technique. The results are means of 2–3 independent experiments ± SE.

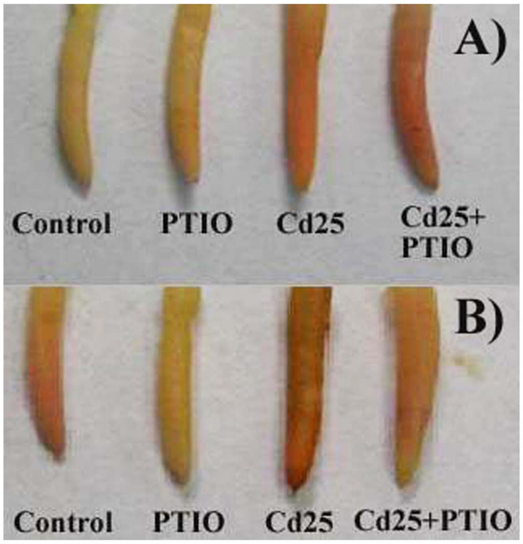

Figure 2. Accumulation of cadmium in the roots of soybean seedlings detected by histochemical staining with Dithizone reagent. The seedlings were treated with distilled water (control), 50 μM PTIO (PTIO), $CdCl_2$ with cadmium in the concentration of 25 mg L-1 (Cd25), or $CdCl_2$ with cadmium in the concentration of 25 mg L-1 and 50 μM PTIO (Cd25+PTIO) for 3 (**A**) or 6 (**B**) hours.

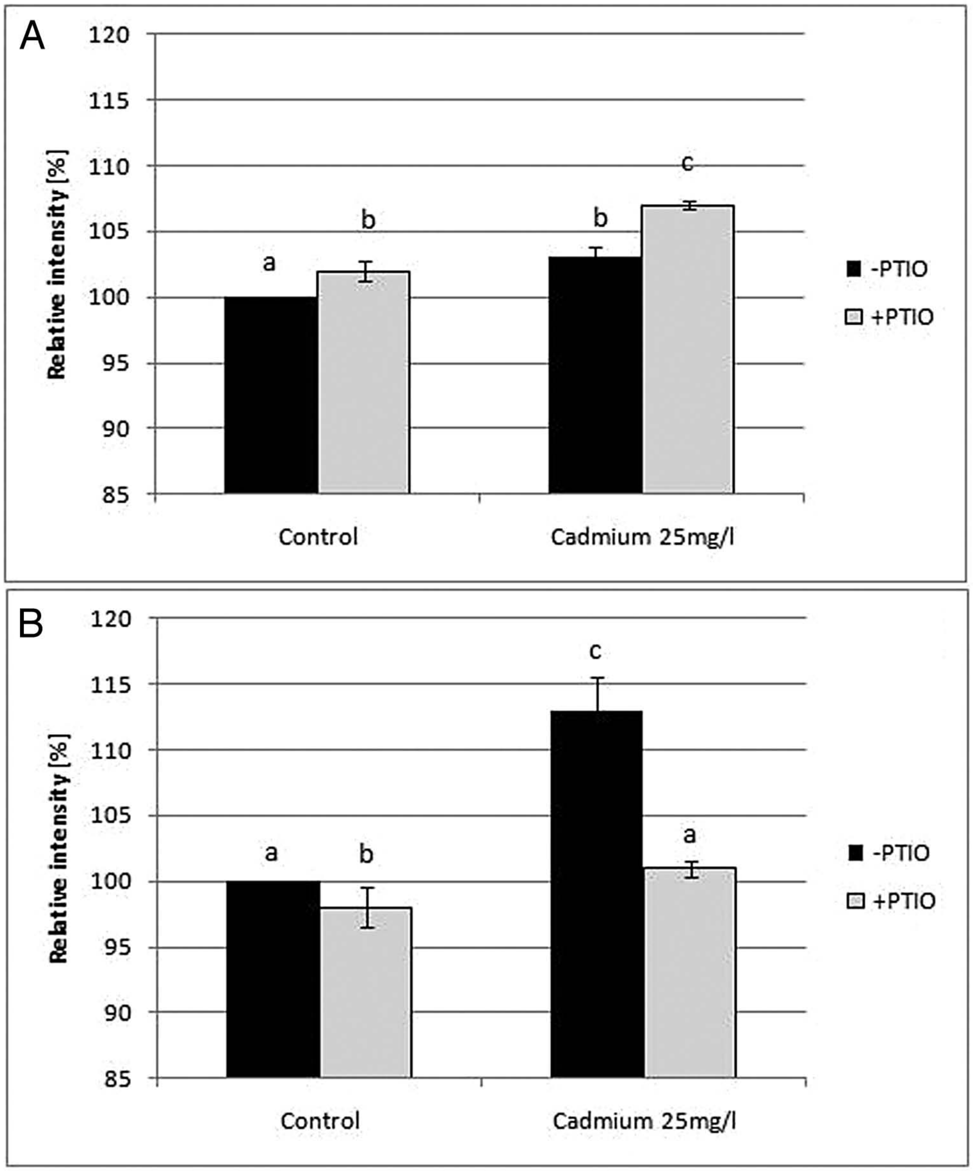

Figure 3. Relative staining intensity of soybean seedlings roots. The seedlings were treated according to the description in **Figure 2** for 3 (**A**) or 6 (**B**) hours. Densitometric quantification was performed with the use of Science Lab Software. The results are means of 2 independent experiments ± SE.

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